UNIVERSITI PUTRA MALAYSIA

SOME BIOGENIC AMINES IN THE PRAWN HAEMOLYMPH DURING OVARIAN GROWTH AND THEIR EFFECTS ON VITELLIN BIOSYNTHESIS IN THE PENAEID PRAWN, PENAEUS MERGUEINSIS, DEMAN

CHONG HUEY BING

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By

CHONG HUEY BING

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in Faculty of Medicine and Health Sciences Universiti Putra Malaysia

June 2000

Specially dedicated to

MY PARENTS

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

SOME BIOGENIC AMINES IN THE PRAWN HAEMOLYMPH DURING OVARIAN GROWTH AND THEIR EFFECTS ON VITELLIN BIOSYNTHESIS IN THE PENAEID PRAWN, *PENAEUS MERGUIENSIS*, DE MAN

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Chairperson: **Chan Hooi Har, Ph.D.**

Faculty: **Medicine and Health Sciences**

A study on the biogenic amines in the haemolymph of penaeid prawn, *Penaeus merguiensis*, at different stages of ovarian growth was conducted. The gonadosomatic index (GSI) was used to show the stages of the ovarian growth. The qualitative and quantitative determination of the biogenic amines were accomplished by using the high performance liquid chromatography with electrochemical detection (HPLC-ECD) method, which provides a high sensitivity and selectivity in determining the biogenic amines. The biogenic amines can be detected at the picogram levels using this method.

The haemolymph of *P. merguiensis* at the immature stage (GSI 0.1-4.0) contained the highest level of 5-hydroxyindole acetic acid (5-HIAA) (250 pg/mg). It decreased

at the maturing stage (GSI 4.1-8.0) and increased again at the mature stage (GSI 8.1-12.0). Both norepinephrine (NE) and dopamine (DA) were not detected in the haemolymph at the immature stage. The level of adrenaline (AD) was found to be in increasing order from immature (10 pg/mg) to maturing (20 pg/mg) and mature (140 pg/mg) stages. The concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) increased from immature (60 pg/mg) to maturing (120 pg/mg) stages, but decreased at the mature stage (100 pg/mg). Neither 5-hydroxytryptamine (5-HT) nor 5-methoxytryptamine (5-MT) were detected in the haemolymph samples of *P. merguiensis* in the present study.

An unknown compound was detected in the haemolymph of *P. merguiensis* at different ovarian growth stages. The levels of this unknown compound decreased from immature to maturing and mature stages, implying that it might be needed for the ovarian growth processes.

The effectiveness of some selected biogenic amines, 5-HT, 5-HIAA and melatonin in stimulating *in vitro* vitellin biosynthesis in the ovarian tissues of *P. merguiensis* were studied. Combinations of 5-HT with brain (BR) and 5-HT with thoracic ganglion (TG) treatments significantly ($P < 0.001$) induced vitellin biosynthesis. Ovarian tissues incubated with 5-HT in the absence of brain and thoracic ganglion did not significantly ($P > 0.05$) stimulate the vitellin biosynthesis in the ovarian tissues of *P. merguiensis*. The treatments with 5-HIAA or melatonin itself significantly ($P < 0.05$) induce vitellin biosynthesis as compared to the control.

However, the results of these treatments were insignificant ($P > 0.05$) when compared with the BR and TG treatments.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains.

BEBERAPA BIOAMINA DALAM HEMOLIMF UDANG SEMASA PERKEMBANGAN OVARI DAN KESANNYA TERHADAP BIOSINTESIS VITELIN PADA UDANG LAUT, *PENAEUS MERGUIENSIS*, DE MAN

Oleh

CHONG HUEY BING

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Satu kajian terhadap bioamina dalam sampel hemolimf dari udang laut, *Penaeus merguiensis* pada peringkat perkembangan ovari yang berlainan telah dijalankan. Indeks gonadosomatik (gonadosomatic index, GSI) menunjukkan peringkat perkembangan ovari udang laut. Kehadiran bioamina-bioamina ditentukan melalui teknik "high performance liquid chromatography with electrochemical detection" (HPLC-ECD). Teknik ini dapat memberi kepekaan dan kuasa pemilihan yang tinggi terhadap bioamina yang diselidiki. Bioamina-bioamina tersebut dapat dikesan sehingga ke takat pikogram.

Keputusan kajian menunjukkan bahawa sampel hemolimf *P. merguiensis* pada peringkat tidak matang (immature) (GSI 0.1-4.0) mengandungi kepekatan

5-hydroxyindole acetic acid (5-HIAA) yang paling tinggi (250 pg/mg). Ia menurun pada peringkat mencapai kematangan (maturing) (GSI 4.1-8.0) dan meningkat semula pada peringkat matang (mature) (GSI 8.1-12.0). Kedua-dua jenis bioamina iaitu norepinephrine (NE) dan dopamine (DA) tidak dapat dikesan pada peringkat tidak matang. Kepekatan adrenaline (AD) meningkat sepanjang peringkat perkembangan ovari manakala kepekatan 3,4-dihydroxyphenylacetic acid (DOPAC) meningkat dari peringkat tidak matang (60 pg/mg) ke peringkat mencapai kematangan (120 pg/mg), tetapi menurun pada peringkat matang (100 pg/mg). Keputusan kajian ini juga menunjukkan bahawa kesemua sampel hemolimf tidak mengandungi sebarang 5-hydroxytryptamine (5-HT) dan 5-methoxytryptamine (5-MT).

Suatu bahan yang tidak dapat dikenalpasti telah dijumpai dalam hemolimf *P. merguiensis* pada peringkat kematangan ovari yang berlainan. Kepekatan bahan ini menurun sepanjang peringkat kematangan ovari. Ini menunjukkan bahawa bahan ini mungkin diperlukan dalam proses kematangan ovari udang.

Beberapa bioamina seperti 5-HT, 5-HIAA dan melatonin telah dipilih untuk mengkaji keberkesanannya dalam merangsang biosintesis vitelin *in vitro* pada tisu ovari *P. merguiensis*. Kesan yang ketara ($P < 0.001$) dapat dilihat pada rawatan 5-HT bersamaan tisu otak dan 5-HT bersamaan torasik ganglion berbanding dengan kawalan. Rawatan dengan 5-HT tanpa tisu otak dan torasik ganglion tidak menunjukkan kesan yang ketara ($P > 0.05$) dalam peningkatan biosintesis vitelin.

Tisu ovari yang menerima rawatan 5-HIAA atau melatonin sahaja memberi kesan yang ketara ($P < 0.05$) berbanding dengan kawalan. Tetapi, kesan kedua-dua rawatan tersebut adalah tidak ketara ($P > 0.05$) jika dibanding dengan rawatan tisu otak dan torasik ganglion.

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I certify that an Examination Committee met on 20th June 2000, to conduct the final examination of Chong Huey Bing on her Master of Science thesis entitled "Some Biogenic Amines in the Prawn Haemolymph during Ovarian Growth and Their Effects on Vitellin Biosynthesis in the Penaeid Prawn, *Penaeus merguiensis*, de Man" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

CHONG HUEY BING

Date: 24-6-2000

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATE

LIST OF ABBREVIATIONS

AD	Adrenaline
ANOVA	Analysis of Variance
BL	Body length
BR	Brain
BCS	Biodegradable Counting Scintillant
CL	Carapace length
DA	Dopamine
DHBA	3, 4-Dihydroxybenzylamine
DOC	Sodium deoxycholate
DOPA	Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic acid
ECD	Electrochemical detector
EDTA	Ethylene Diamine Tetra-Acetate
GCMS	Gas chromatography-mass spectrometry
GI	Gonadal index
GIH	Gonad inhibiting hormone
GSH	Gonad stimulating hormone
GSI	Gonadosomatic index
HA	Histamine
HCG	Human chorionic gonadotrophin

$HClO_4$	Perchloric acid
5-HIAA	5-Hydroxyindole acetic acid
HPLC	High performance liquid chromatography
HPLC-ECD	High performance liquid chromatography with electrochemical detection
5-HT	5-Hydroxytryptamine
JH	Juvenile hormone
MF	Methyl farnesoate
5-MT	5-Methoxytryptamine
NAT	N-acetyltransferase
NE	Norepinephrine
OA	Octopamine
PAG	Polyacrylamide Gel
PFP	Pentafluoropropionic anhydride
PMSF	Phenylmethylsulfonylfluoride
RIA	Radioimmunoassay
RT	Retention times
SDS	Sodium dodecyl sulphate
TCA	Trichloroacetic acid
TG	Thoracic ganglion
TGE	Thoracic ganglion extract

CHAPTER 1

INTRODUCTION

The shrimp culture industry is one of the most lucrative industries in many countries with aquacultural development, including Malaysia. The large scale production of penaeid shrimps is a recent development, stimulated by a common pattern of increasing demand and on the other hand, by a decline of natural supplies caused by over-fishing, aquatic pollution and diseases.

The spawner (broodstock) is essential for the production of penaeid shrimp postlarvae in penaeid shrimp culture. However, at present in some countries, marine prawn seed supply is still dependent on postlarvae collected from coastal waters. The availability of the spawners has also been mainly dependent on the capture of wild female spawners from the sea. This is because the ovaries of captive females of several penaeid species, for example, *Penaeus monodon* Fabricius, *Penaeus vannamei*, *Penaeus semisulcatus*, and *Penaeus japonicus* do not easily attain sexual maturity without external manipulation. Therefore, development of methods to induce sexual maturation of female shrimps in captivity should be given high priority to assure a stable supply of spawners and seed availability.

Various attempts have been made to stimulate ovarian growth, such as manipulation of diet (Middleditch *et al*., 1979; Nascimento *et al*., 1991), manipulation of environmental conditions (Hillier, 1984; Luis and Ponte, 1993), and hormonal regulation such as eyestalk ablation (Adiyodi and Adiyodi, 1970; Fingerman, 1970; Kleinholz and Keller, 1979; and Charniaux-Cotton, 1985). Among these methods, eyestalk ablation is the most popular and commonly applied. Panouse (1943) first showed that eyestalk ablation induced ovarian maturation in the shrimp, *Leander serratus* (Pennant). This was subsequently confirmed by Primavera (1978) and Emmerson (1983), using other species including *P. monodon*.

Unfortunately, these methods are not totally successful and the results are inconsistent. For example, the unilateral eyestalk ablation method is completely at variance with the natural physiology of the animal, and this manipulation may have a negative effect on the quality of the eggs. Conte *et al*. (1977) found that female shrimps that have undergone unilateral eyestalk ablation suffered from high mortality when placed in net cages in the pond. Browdy and Samocha (1985a, b) showed that there is a decrease in fecundity (as measured by the number of eggs per spawn) for the ablated females.

In order to study the hormonal regulation of crustacean reproduction, it is important to know the specific events and the origin of yolk produced during sexual maturation. Ovarian maturation in crustaceans consists of two major processes: oogenesis and vitellogenesis. During oogenesis, oogonia accumulate glycoproteins to become primary oocytes. When the oocytes reach a diameter typical of the